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ORIGINAL ARTICLE

Niacin Exacerbates Methyl Prednisolone-Induced Bone Changes in Growing Rats

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ABSTRACT

Positive role of niacin on serum lipid profile of rats treated with glucocorticoids (GCs) has been reported. This study aims to evaluate effect of niacin on bone changes in GC-induced dyslipidemic rats. A number of 28 growing rats divided into four groups and treated as control, Methyl prednisolone (MP) group (3.5 mg/kg five days a week, SC); MPN group (MP + niacin 200 mg/kg/day orally) and MPA group (MP + alendronate 0.03 mg/kg/day, SC). After 4 weeks, serum lipid profile and histomorphometric parameters including trabecular width (Tb.Wi), trabecular separation (Tb.Sp) and number (Tb.N), bone area/tissue area (B.Ar/T.Ar) and osteoid thickness (O.Th) in metaphyseal side of growth plate of femoral head were determined. Obvious dyslipidemia and decreased B.Ar/T.Ar and O.Th were observed in MP group. Niacin alleviated dyslipidemia, however MPN rats had appreciably lower Tb.N and higher Tb.Sp as compared to MP group. Alendronate had a moderate positive effect on bone changes. Although niacin effectively ameliorates GC-induced dyslipidemia in growing rats, it may exacerbate bone changes.

Keywords: *Niacin, Dislipidemia, Methyl prednisolone, Bone, Histomorphometry, Rat*

Systemic use of synthetic glucocorticoids (GCs) is indicated in a wide variety of disorders, including autoimmune, pulmonary and gastrointestinal diseases, as well as in patients following organ transplantation and those with malignancies. However; their use may be limited by their potential side effects including development of osteoporosis and consequent fractures [1]. In fact, the most common secondary form of osteoporosis is that induced by GCs, which predominantly affects regions of the skeleton with abundant cancellous bone such as lumbar spine and proximal femur [2]. On the other hand, it has been generally perceived that GCs adversely affect serum lipid levels [3]. In fact, GC administration is associated with serum lipid disturbances including elevations in total cholesterol, triglycerides, LDL-c and HDL-c in humans [4] as well as laboratory animals [5,6].

Niacin (nicotinic acid or vitamin B_3) can induce beneficial changes in serum lipoproteins and its use has been associated with beneficial cardiovascular effects. Niacin reduces low-density lipoprotein, increases high-density lipoprotein, and decreases triglycerides [7]. In 2012, Safaei et al., demonstrated that niacin ameliorates lipid disturbances due to GC administration in rats [6]. Unfortunately, few reports of animal experiments declare that niacin especially at high doses may adversely affect bone [8,9]. Regarding the positive effects of niacin on GC-induced dyslipidemia and the potential of its use in GC-treated patients who are at risk for development of osteoporosis and bone loss, the present study evaluates the effects of anti hypercholesterolemic dosage of niacin on methyl prednisolone (MP)-induced bone changes in growing rats by using a histomorphometric approach.

MATERIALS AND METHODS

Animals and experimental design

Twenty eight female Sprague-Dawley rats, about three weeks old with a mean body weight of 220 g, were purchased from animal house of Shiraz Medical

Table 1. Serum lipid parameters presented as mean ± SD in different groups (n= 7) at the end of the experiment

Parameters	Groups			
	Control	**MP**	**MPN**	**MPA**
Total cholesterol (mg/dl)	40 ± 6.4	94 ± 6.6*	57 ± 9.9*,#	45 ± 2.7#
Triglycerides (mg/dl)	54 ± 10.1	232 ± 63.2*	100 ± 19.5#	59 ± 11.6#
LDL-c (mg/dl)	22 ± 11	101 ± 18.9*	43 ± 17.3#	17 ± 5#
VLDL-c (mg/dl)	10 ± 2	46 ± 12.7*	20 ± 3.9#	11.9 ± 2.3#
HDL-c (mg/dl)	21 ± 3.6	25 ± 5.3	40 ± 7.6*	49 ± 10*,#

*and # signs are used to demonstrate significant differences with control and MP groups respectively ($p < 0.001$).
Control: normal saline; MP: methyl prednisolone; MPN: methyl prednisolone+niacin and MPA: methyl prednisolone+alendronate.

University, Shiraz, Iran. Rats were acclimatized for one week before the beginning of the experiment to the ambient conditions (temperature about 23°C and a 12h/12h, light/dark cycle). Animals had free access to tap water and standard rat chow diet prepared by Razi Vaccine and Serum Research Institute, Shiraz, Iran. After adaptation, rats were randomly allocated into four equal groups (n = 7 each) and treated as follows for 4 weeks: group 1 (control): normal saline; group 2 (MP): MP acetate (Aburaihan pharmaceutical Co., Tehran, Iran), 3.5 mg/kg five days a week, SC; group 3 (MPN): MP acetate, 3.5 mg/kg five days a week, SC + niacin (Novin Kavosh Mamtir Co., Tehran, Iran) 200 mg/kg daily by oral gavages and group 4 (MPA): MP acetate, 3.5 mg/kg five days a week, SC + sodium alendronate (Aburaihan pharmaceutical Co., Tehran, Iran), 0.03 mg/kg daily, SC.

Procedures used in the present study are in accordance with institutional ethical guidelines of School of Veterinary Medicine, Shiraz University, for care and use of laboratory animals in experiments.

Determination of serum lipid levels

At the end of the experiment, rats were fasted over night and blood samples were collected under chloroform anesthesia by cardiac puncture. After centrifugation at 2000 rpm for 20 min, harvested sera were stored in -70°C until use. Serum total cholesterol, triglycerides and HDL-c were assayed by commercial colorimetric kits prepared by Ziest Chem ® Diagnostics, Tehran, Iran (total cholesterol and triglycerides) and Azma Teb Sahand Co., Tehran, Iran (HDL-c). LDL-c and VLDL-c were calculated according to Friedwald equation.

Histomorphometric study of bone

After blood collection, rats were euthanized by deepening anesthesia and left femoral bones were dissected for histomorphometric study. Bones were fixed in 4% formaldehyde solution and decalcified using formic acid–sodium Citrate method [10]. Then 5-µm longitudinal sections of femoral head and neck were made in the median plate. Sections were stained using Masson's trichrome method [10]. Histomorphometric parameters were determined by a digital photo microscope connected to a personal computer with Ziess axio vision LE software. Parameters which measured in secondary spongiosa of metaphyseal side of growth plate in femoral head; included trabecular width (Tb.Wi), trabecular separation (Tb.Sp) and number (Tb.N), bone area/tissue area (B.Ar/T.Ar) and osteoid thickness (O.Th). The region of the cancellous bone marked for the measurements was the central zone of cancellous tissue. The nomenclature of parameters is in compliance with ASBMR histomorphometry nomenclature committee [11].

Statistical analysis

Data were presented as mean ± SD. Data analysis was carried out by using one-way ANOVA and Tukey's multiple comparison tests as the *post hoc* (SPSS 11.5 for windows software). Differences were considered significant at $p < 0.05$.

RESULTS

Serum lipid levels

Obvious hypercholesterolemia and hypertriglyceridemia accompanied by increased levels of LDL-c and VLDL-c were present in MP group. All these parameters were significantly lower in MPN as well as MPA rats as compared to MP group. A slight increase in HDL-c level was observed in MP rats in comparison with control group. Rats of MPN and MPA groups had significantly higher levels of HDL-c in comparison with control rats. Results are summerized in Table 1.

Bone histomorphometric parameters

As demonstrated in Table 2, no significant difference observed in Tb.Wi of different groups. B.Ar/T.Ar as well as O.Th significantly decreased in rats from MP group as compared to control rats. These parameters in MPN rats were statistically the same as MP group. Whereas MP administration alone had no significant effect on Tb.N and Tb.Sp; MPN rats had

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Table 2. Histomorphometric parameters of metaphyseal side of growth plate in femoral head presented as mean ± SD in different groups (n=7) at the end of the experimen

Parameters	Groups			
	Control	MP	MPN	MPA
Tb.Wi (μm)	38 ± 5.4	33 ± 4.8	41 ± 5	40 ± 3.1
B.Ar/T.Ar (%)	53 ± 4.9	38 ± 6.7*	39 ± 6.2*	48 ± 6.6
Tb.N (1/mm)	13 ± 2	11 ± 2.1	8 ± 1.7*,#	12 ± 1.6
Tb.Sp (μm)	37 ± 7.3	58 ± 11	85 ± 16*,#	44 ± 11
O.Th (μm)	6.4 ± 0.2	5.2 ± 1*	6 ± 1	5.7 ± 0.6

*and # signs are used to demonstrate significant differences with control and MP groups respectively ($p < 0.05$).
Control: normal saline; MP: methyl prednisolone; MPN: methyl prednisolone+niacin and MPA: methyl prednisolone+alendronate.
Tb.Wi: trabecular width; B.Ar/T.Ar: bone area/tissue area; Tb.N: trabecular number; Tb.Sp: trabecular separation and O.Th: osteoid thickness.

appreciably lower Tb.N and higher Tb.Sp as compared to both control and MP groups. Data from MPA rats were statistically the same as both control and MP rats for all parameters.

DISCUSSION

The aim of the present study was to investigate the effects of niacin administeration at its anti hypercholesterolemic dosage on MP-induced bone loss in skeletally-growing rats. Rats reach their sexual maturity at the age of about 2.5 months, however their skeleton is considered mature after the age of 10 months [12]. Skeletally-immature rats have a low peak bone mass and are an appropriate animal model in the research of endocrine, nutritional and environmental factors [13]. The establishment of bone loss in the present study due to GC administration was confirmed by the reduction of B.Ar/T.Ar which indicates a bone deficit in cancellous bone mass. Moreover, O.Th as a static remodeling parameter was reduced in rats treated with GC. Histomorphometric studies performed by Nitta *et al.* (1999) [14] demonstrated that the most characteristic consequence of MP treatment in rats is a significant loss of trabecular bone resulting from thinning of trabecular bone without changing the connectivity. In our study, only a slight decrease was observed in Tb.Wi of MP treated rats as compared to control which may be described by relatively short duration of the experiment. Alendronate which was used as a comparative control in our study, had a moderate improving effect since all bone parameters in this group were statistically the same as both MP and control groups. Regarding serum lipid profile, consistent with previous studies [4-6] a characteristic GC-induced dyslipidemia manifested as hypercholesterolemia, hypertriglyceridemia and increased levels of LDL-c, HDL-c and VLDL-c was present in MP treated rats at the end of the experiment. Both niacin and alendronate had appreciable positive effects on serum lipid profile which was consistent with some previous studies [6,15].

The use of some hypolipidemic agents has been associated with positive effects on bone. It has been reported that HMG-CoA reductase inhibitors (statins) can increase bone mineral density in postmenopausal women [16] and stimulate bone formation *in vitro* and *in vivo* [17]. In the rabbit, lipid-lowering agents from statin (lovastatin) and non-statin (bezafibrate) categories have alleviated steroid-induced osteoporosis with the same degree [18]. Ruan et al. (2012) suggest that bone anabolism regulated by statins may be attributable to three aspects: promotion of osteogenesis, inhibition of osteoblast apoptosis and suppression of osteoclastogenesis [19]. Inhibition of 3-hydroxy-3-methyl glutaryl-CoA reductase in osteoblasts by statins has a major role in promoting osteogenesis and osteoblast differentiation [20-22] and direct effect of statins on blood lipid profile is not involved in this regard. Moreover, bezafibrate can directly stimulate proliferation and differentiation of cultured osteoblastic MC3T3-E1 cells, mainly via a PPARβ-dependent mechanism [23]. As mentioned previously, in the present study, niacin administered in its lipid-lowering dose although effectively ameliorated GC-induced dyslipidemia, did not reveal favorable effects on bone histomorphometric parameters (except for a moderate effect on O.Th) and even exacerbated the effect of MP on number and separation of trabeculae in metaphyseal side of growth plate of femoral head. It seems that mechanisms behind the effect of niacin on bone are not directly related to its lipid lowering properties and other pathways may be involved in this situation. Niacin induces a receptor-mediated increase in both PGD_2 and PGE_2 generation in a dose-dependent manner in THP-1 macrophages and Langerhans dendritic cells by causing an increase in cytosolic calcium and activation of cytosolic phospholipase A_2 [24]. On the other hand, in bone resorption associated with inflammation, PGE_2 is mainly produced by osteoblasts and acts as a potent stimulator of bone resorption [25]. Although speculative, niacin-induced increase in PGE_2 synthesis by osteoblasts may be involved in deteriorative effects

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of niacin on bone parameters observed in our study. This preliminary assumption needs to be investigated in future studies.

In conclusion, our findings demonstrate that although niacin effectively ameliorates GC-induced dyslipidemia, it has no significant positive effect on bone changes and even adversely affects some structural bone parameters including TB.N and Tb. Sp in growing rats.

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